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(54) Title: POLY(ADP-RIBOSE) POLYMERASE INHIBITORS TO TREAT DISEASES ASSOCIATED WITH CELLULAR SENESCENCE

(57) Abstract

Inhibition of the enzyme poly(ADP-ribose) polymerase can delay the onset of senescence and inhibitors of the enzyme can be used to treat diseases caused or exacerbated by cellular senescence.

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## Title

Poly(ADP-Ribose) Polymerase Inhibitors to treat Diseases Associated with Cellular Senescence

## Background of the Invention

### Field of the Invention

The present invention relates to the fields of molecular biology, gerontology, and medical pharmacology and diagnostics.

### Description of Related Art

There is substantial evidence that somatic cells have a finite replicative capacity (Hayflick and Moorhead, 1961, *Exp. Cell Res. 25*: 585-621; Hayflick, 1965, *Exp. Cell Res. 37*: 614-636; and Hayflick, 1970; *Exp. Geront. 5*: 291-303) and that this process is a major etiological factor in aging and age-related disease (Goldstein, 1990, *Science 249*: 1129-1133; Stanulis-Praeger, 1987, *Mech. Ageing Dev. 38*: 1-48; and Walton, 1982, *Mech. Ageing Dev. 19*: 217-244). As cells undergo replicative senescence *in vitro* and *in vivo*, the cells not only lose the ability to divide in response to growth stimuli, but there are also significant deleterious changes in the pattern of gene expression (West, 1994, *Arch. Derm. 130*: 87-95). As an individual grows older, senescent cells make up an increasing percentage of the cells present in the tissues of the aging individual. The altered pattern of gene expression exhibited by senescent cells is likely to contribute significantly to age-related pathologies. Reversal of, or a delay in the onset of, senescence should provide an effective therapy for diseases in which replicative senescence plays a role.

There is growing evidence that the fundamental cause of cellular senescence is the progressive loss of telomeric repeated DNA in somatic cells that lack the enzyme designated telomerase (see Harley, 1991, "Telomere loss: Mitotic clock or genetic time bomb?" *Mut. Res.* 256:271-282). There is currently no consensus as to the molecular mechanisms that recognize the shortened telomeres in aged cells and cause a cell cycle arrest at the $G_1$/S interface, but this arrest may be caused by a DNA checkpoint arrest in which the senescent cell recognizes the shortened telomere as damaged DNA and causes cell cycle arrest similar to that observed in normal cells, which arrest their growth in the presence of DNA damage.

The mammalian enzyme Poly (ADP-Ribose) Polymerase (PADPRP) has been implicated in the signaling of DNA damage. PADPRP activity is higher in isolated nuclei of SV40-transformed fibroblasts than in those of untransformed fibroblasts; leukemic cells show higher enzyme activity than normal leukocytes; and colon cancers show higher enzyme activity than normal colon mucosa (see Miwa *et al.*, 1977, *Arch. Biochem. Biophys. 181*: 313-321; Burzio *et al.*, 1975, *Proc. Soc. Exp. Biol. Med. 149*:933-938; and Hirai *et al.*, 1983, *Cancer Res. 43*:3441-3446. These observations led to the conclusion that the enzyme activity responds to DNA damage and parallels DNA repair. Supporting this conclusion is the observation that the reduction of the activity of the enzyme by certain drugs increases DNA amplification and consequent oncogenesis in cells (see Harris, 1985, *Int. J. Radiat. Biol. 48*: 675-690).

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More recent work has focused on the mechanism by which PADPRP modulates DNA replication and repair (see Smulson and Sugimura, eds., "Novel ADP-ribosylations of regulatory enzymes and proteins," Elsevier, N.Y. (1980)). Such studies have identified PADPRP as an ~113 kDa protein that uses NAD as a substrate in the formation of poly (ADP-ribose) chains at sites on many nuclear proteins. The enzyme binds tightly to DNA and requires DNA strand breaks for activity (see Benjamin and Gill, 1980, *J. Biol. Chem. 255*: 10502-10508). The PADPRP enzyme system appears to function in response to transient and localized DNA strand breaks in cells that may arise through a variety of processes including DNA repair, replication, recombination, and gene rearrangement (see Alkhatib *et al.*, 1987, *Proc. Natl. Acad. Sci. USA 84*:1224-1228). The cDNA corresponding to the PADPRP gene has also been cloned and sequenced (see Cherney *et al.*, 1987, *Proc. Natl. Acad. Sci. USA 84*:8370), and methods for detecting a predisposition to cancer arising out of mutations in the PADPRP gene have been reported (see U.S. Patent No. 5,272,057).

Inhibitors of PADPRP have also been developed, primarily for the purpose of enzymatic studies (see Banasik *et al.*, 1992, *J. Biol. Chem. 267*: 1569-1575) and for use in cancer and anti-viral therapies (see PCT patent publication No. 91/18591). PADPRP inhibitors have been reported to be effective in radiosensitizing hypoxic tumor cells (see U.S. Patent Nos. 5,032,617; 5,215,738; and 5,041,653). These compounds can also be used to prevent tumor cells from recovering from potentially lethal damage of DNA after radiation therapy, presumably by their ability to prevent DNA repair.

One weak inhibitor of PADPRP known as kinetin (Althaus, F.R., and Richter, C., 1987, "ADP-ribosylation of Proteins" (Springer-Verlag); see p. 26) has also been reported to delay the onset of aging characteristics in human fibroblasts (see Rattan and Clark, 1994, Biochem. Biophys. Res. Comm. 201(2): 665-672). However, the researchers speculated that kinetin acted through receptor-mediated action on the components of protein synthetic machinery, improving the efficiency of various maintenance and repair pathways such as fidelity of protein synthesis, scavenging free radicals, and removing abnormal and damaged macromolecules. Moreover, the researchers stated that the anti-aging effects of kinetin were not accompanied by an increase in cell culture lifespan in terms of maximum proliferative capacity *in vitro*.

Consequently, there remains a need for compounds that can delay the onset of senescence and extend the maximum proliferative capacity of cells *in vivo* and *in vitro*. The present invention meets this and other needs.

## Summary of the Invention

In a first aspect, the present invention provides a method to extend the lifespan and proliferative capacity of cells, which method comprises administering a therapeutically effective amount of a PADPRP inhibitor to said cells under conditions such that PADPRP activity is inhibited. The method is especially useful in treating disease or disease conditions induced or exacerbated by cellular senescence. In particular, the method of the invention is useful in treating

skin aging, Alzheimer's disease, atherosclerosis, osteoarthritis, osteoporosis, age-related macular degeneration, Duchenne muscular dystrophy or other degenerative diseases of skeletal muscle involving replicative senescence, and immune senescence, including diseases, such as AIDS, that result in immune senescence.

In a second aspect, the present invention provides a method to alter gene expression of senescent cells, which method comprises administering a therapeutically effective amount of a PADPRP inhibitor to said cells under conditions such that PADPRP activity is inhibited. Senescent gene expression can be altered by increasing expression of young cell specific genes and/or decreasing expression of senescent cell specific genes. This method is particularly useful in treating diseases and conditions associated with cellular senescence and aging.

In a third aspect, the present invention provides compounds and formulations useful in the above methods. Preferred compounds for use in the present method include 3-hydroxybenzamide, 3-acetamidobenzamide, 3-methoxybenzamide, 3-methylbenzamide, 3-fluorobenzamide, 2-methoxybenzamide, 3-chlorobenzamide, benzamide, 4-amino-1,8-naphthalimide, 2H-benz[c]isoquinolin-1-one [6(5H)-phenanthridinone], 2-nitro-6(5H)-phenanthridinone, 1,5-dihydroxyisoquinoline, 2H-benz[de]isoquinoline-1,3-dione, (1,8-naphthalimide), 2-methyl-4(3H)-quinazolinone, 1-hydroxyisoquinoline (isocarbostyril), 2,4(1H,3H)-quinazolinedione (benzoyleneurea), chlorthenoxazin, 4-hydroxyquinazoline, 1(2H)-phthalazinone, 2-phenylchromone (flavone), 3-aminophthalhydrazide (luminol), N-formyl luminol, arachidonic acid, oleic acid, linoleic acid, and nicotinamide.

These and other aspects of the invention are described in more detail below, beginning with a brief description of the accompanying drawings.

## Brief Description of the Drawings

Figure 1 shows the results of treating human fibroblast cells (W138 cells) *in vitro* with varying concentrations of 3-aminobenzamide (3-AB) as measured in the maximum achievable cumulative population doublings. The X-axis shows the number of days in culture, while the Y-axis shows the cumulative population doublings observed for each of the treated cell cultures. A significant increase in the maximum achievable cumulative population doublings was observed with cells treated with 1 mM 3-AB.

Figure 2 shows the results of treating W138 cells with varying concentrations of 1, 5-dihydroxyisoquinoline (1, 5-DHI; also known as 1, 5-isoquinolinediol). The X-axis shows the number of days in culture, and the Y-axis shows the maximum achievable cumulative population doublings. At 100 μM (DMSO concentration 0.1%), the cells displayed an extension of their proliferative lifespan.

## Description of the Preferred Embodiments

The present invention provides a method to extend the lifespan and proliferative capacity of cells, which method comprises administering a therapeutically effective amount of a PADPRP inhibitor to said cells under conditions such that PADPRP activity is inhibited. The method is especially useful in treating disease or disease conditions induced or exacerbated by cellular senescence. In particular, the method of the invention is useful in treating skin aging, Alzheimer's disease, atherosclerosis, osteoarthritis, osteoporosis, muscular dystrophy, age-related macular degeneration, and immune senescence, including diseases, such as AIDS, that result in immune senescence.

Compounds for use in the present method include any compound that specifically inhibits PADPRP, such as those disclosed in Banasik *et al.*, 1992, *J. Biol. Chem.* 267: 1569-1575, and U.S. Patent Nos. 5,032,617; 5,215,738; and 5,041,653. Preferred compounds of the invention are shown in Table 1, below.

Table 1

PADPRP Inhibitors

| Compound | $IC_{50}$ (μM) |
|---|---|
| Benzamide analogues: | |
| 3-Hydroxybenzamide | 9.1 |
| 3-Acetamidobenzamide | 12 |
| 3-Methoxybenzamide | 17 |
| 3-Methylbenzamide | 19 |
| 3-Fluorobenzamide | 20 |
| 2-Methoxybenzamide | 20 |
| 3-Chlorobenzamide | 22 |
| Benzamide | 22 |
| 3-Aminobenzamide | 33 |
| 5-Acetamidosalicylamide | 45 |
| m-Phthalamide (isophthalamide) | 50 |
| 3-Bromobenzamide | 55 |
| 2-Hydroxybenzamide (salicylamide) | 82 |
| Fatty Acids: | |
| Arachidonic acid | 44 |
| Linoleic acid | 48 |
| Oleic acid | 82 |

Other Compounds:

| | |
|---|---|
| 4-amino-1.8-naphthalimide | 0.18 |
| 2H-Benz[c]isoquinolin-1-one [6(5H)-phenanthridinone] | 0.30 |
| 2-Nitro-6(5H)-phenanthridinone | 0.35 |
| 1.5-Dihydroxyisoquinoline | 0.39 |
| 2H-Benz[de]isoquinoline-1.3-dione (1.8-naphthalimide) | 1.4 |
| 2-Methyl-4(3H)-quinazolinone | 5.6 |

Table 1

PADPRP Inhibitors (cont.)

| | |
|---|---|
| 1-Hydroxyisoquinoline (isocarbostyril) | 7.0 |
| 2.4(1H.3H)-Quinazolinedione (benzoyleneurea) | 8.1 |
| Chlorthenoxazin | 8.5 |
| 4-Hydroxyquinazoline | 9.5 |
| 1(2H)-Phthalazinone | 12 |
| 2-Phenylchromone (flavone) | 22 |
| 3-Aminophthalhydrazide (luminol) | 23 |
| 2.3-Dihydro-1.4-phthalazinedione (Phthalhydrazide) | 30 |
| 5-Iodouridine | 43 |
| 2-Mercapto-4(3H)-quinazolinone | 44 |
| 2-Methyl-1.4-benzopyrone (2-methylchromone) | 45 |
| 5-Iodouracil | 71 |
| 3-Nitrophthalhydrazide | 72 |
| 4-Hydroxy-2-methylquinoline | 74 |
| 4-Hydroxyquinoline | 80 |
| Nicotinamide | 210 |

Preferred compounds of the invention include 3-aminobenzamide and 1, 5-dihydroxyisoquinoline.

To demonstrate the effectiveness of the present method for extending the proliferative capacity and lifespan of cells, human fibroblast cells lines (either W138 at Population Doubling (PDL) 23 or BJ cells at PDL 71) were thawed and plated on T75 flasks and allowed to grow in normal medium (DMEM/M199 plus 10% bovine calf serum) for about a week, at which time the

cells were confluent, and the cultures were therefore ready to be subdivided. At the time of subdivision, the media was aspirated, and the cells were rinsed with phosphate buffered saline (PBS) and then trypsinized. The cells were counted with a Coulter counter and plated at a density of $10^5$ cells per $cm^2$ in 6-well tissue culture plates in DMEM/199 medium supplemented with 10% bovine calf serum and varying amounts (0, 10 µM, 100 µM, and 1 mM; from a 100X stock solution in DMEM/M199 medium) of a PADPRP inhibitor (3-aminobenzamide, purchased from Sigma). This process was repeated every ~7 days until the cells appeared to stop dividing.

The results are shown in Figure 1. As can be seen from the Figure, untreated (Control) cells reached senescence and stopped dividing after about 40 days in culture. While no effect was observed using 10 µM 3-AB, cells treated with 100 µM 3-AB did appear to have a longer lifespan than control cells, and cells treated with 1 mM 3-AB showed a dramatic increase in lifespan and proliferative capacity. The cells treated with 1 mM 3-AB were still dividing after 60 days in culture, a remarkable effect as compared with control cells. In a second example of the method, the results of which are shown in Figure 2, the same procedure was conducted using the PADPRP inhibitor 1, 5-dihydroxyisoquinoline, and the results again show that the treated cells had increased proliferative capacity.

The invention also provides a method to alter gene expression of senescent cells, which method comprises administering a therapeutically effective amount of a PADPRP inhibitor to said cells under conditions such that PADPRP activity is inhibited. PADPRP inhibitors can be used to normalize the expression of those genes for which expression is altered in senescence and the altered expression of which contributes to age-related pathologies and so can be used to treat age related diseases and conditions.

Senescent gene expression can be altered by increasing the expression of young cell specific genes and/or decreasing expression of senescent cell specific genes. These cell specific genes are collectively refered to as "senescence-related genes". A "senescence-related gene" refers to a gene that is expressed at a different level in a senescent cell than in a non-senescent cell of the same cell type.

Northern analysis of the mRNA populations of cell populations, i.e., compound treated senescent cells and nontreated senescent cells, can be employed to examine the effects of a PADPRP inhibitor on panels of genes that show altered expression or abundance in senescence. Various techniques known to those of skill in the art can be used to identify senescence related genes and/or gene products, such as Enhanced Differential Display (EDD) described in U.S. Patent No. 5,580,726, issued Dec. 3, 1996; and/or other techniques described in PCT Pub. No. 96/13610, published May 9, 1996, both of which are incorporated herein by reference. Alternatively, the effects of a PADPRP inhibitor on gene expression in senescent cells can be monitored by immunohistochemistry and these assays can be used to select optimal compounds among a family of PADPRT inhibitors.

Thus, the methods of the invention can be used to increase the lifespan of cells *in vitro* and alter gene expression of senescent cells. While this aspect of the invention is important and of

value for a wide variety of applications in cell culture methodology. a perhaps more important application of the present method involves the treatment of human and other disease. Because cell senescence is implicated in a wide variety of diseases. and may be proven to have a role in essentially all diseases affecting the aged. the present invention offers remarkable promise in the treatment of disease.

In general. a suitable effective dose of a compound of the invention will be in the range of 0.001 to 100 milligram (mg) per kilogram (kg) of body weight of recipient per day. preferably in the range of 0.1 to 10 mg per kg of body weight per day. The desired dosage is preferably presented in one. two. three. four. or more subdoses administered at appropriate intervals throughout the day. These subdoses can be administered as unit dosage forms. for example. containing 5 to 10.000 mg. preferably 10 to 1000 mg of active ingredient per unit dosage form.

The composition used in these therapies can be in a variety of forms. These include. for example. solid. semi-solid. and liquid dosage forms. such as tablets, pills. powders. liquid solutions or suspensions. liposomes. and injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and adjuvants. as is well known to those of skill in the art. See. *e.g.*, *Remington's Pharmaceutical Sciences*. Mack Publishing Co.: Easton. PA. 17th Ed. (1985). Generally. administration will be by oral or parenteral (including subcutaneous. intramuscular. intravenous. and intradermal) routes. The therapeutic methods and agents of this invention can of course be used concomitantly or in combination with other methods and agents for treating a particular disease or disease condition.

While it is possible to administer the active ingredient of this invention alone. it is preferable to present it as part of a pharmaceutical formulation. The formulations of the present invention comprise at PADPRP inhibitor of this invention in a therapeutically or pharmaceutically effective dose together with one or more pharmaceutically or therapeutically acceptable carriers and optionally other therapeutic ingredients. Various considerations are described. *e.g.*, in Gilman *et al.* (eds.) (1990) *Goodman and Gilman's: The Pharmacological Bases of Therapeutics*. 8th Ed.. Pergamon Press: and Remington's supra. each of which is incorporated herein by reference. Methods for administration are discussed therein. *e.g.*, for oral, intravenous. intraperitoneal. or intramuscular administration. and others.

The pharmaceutical compositions will be administered by topical. parenteral. or oral administration for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example. unit dosage forms suitable for oral administration include powder. tablets. pills. and capsules.

Topical administration typically involves the delivery of a PADPRP inhibitor for percutaneous passage of the drug into the systemic circulation of the patient. The skin sites include anatomic regions for transdermally administering the drug as represented by the forearm. abdomen. chest. back. buttock. mastoidal area and the like The PADPRP inhibitor is

administered to the skin by placing on the skin a topical formulation comprising the PADPRP inhibitor or a transdermal drug delivery device that administers the drug, and which bandage is designed, shaped, sized, and adapted for easy placement and comfortable retention on the skin.

A variety of transdermal drug delivery devices can be employed with the compounds described herein. For example, a simple adhesive patch comprising a backing material and an acrylate adhesive can be prepared. The drug and any penetration enhancer can be formulated into the adhesive casting solution. The adhesive casting solution can be cast directly onto the backing material or can be applied to the skin to form an adherent coating. See, *e.g.*, U.S. Patent Nos. 4,310,509, 4,560,555, and 4,542,012.

In other embodiments, the PADPRP inhibitor will be delivered using a liquid reservoir system drug delivery device. These systems typically comprise a backing material, a membrane, an acrylate based adhesive, and a release liner. The membrane is sealed to the backing to form a reservoir. The drug and any vehicles, enhancers, stabilizers, gelling agents, and the like are then incorporated into the reservoir. See, *e.g.*, U.S. Patent Nos. 4,597,961, 4,485,097, 4,608,249, 4,505,891, 3,843,480, 3,948,254, 3,948,262, 3,053,255, and 3,993,073.

Matrix patches comprising a backing, a drug/penetration enhancer matrix, a membrane, and an adhesive can also be employed to deliver PADPRP inhibitors transdermally. The matrix material typically will comprise a polyurethane foam. The drug, any enhancers, vehicles, stabilizers, and the like are combined with the foam precursors. The foam is allowed to cure to produce a tacky, elastomeric matrix which can be directly affixed to the backing material. See, *e.g.*, U.S. Patent Nos. 4,542,013, 4,460,562, 4,466,953, 4,482,534, and 4,533,540.

Also included within the invention are preparations for topical application to the skin comprising a PADPRP inhibitor, typically in concentrations in the range of from about 0.001% to 10%, together with a non-toxic, pharmaceutically acceptable topical carrier. These topical preparations can be prepared by combining an active ingredient according to this invention with conventional pharmaceutical diluents and carriers commonly used in topical dry, liquid, and cream formulations. Ointment and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Such bases may include water and/or an oil such as liquid paraffin or a vegetable oil such as peanut oil or castor oil. Thickening agents which may be used according to the nature of the base include soft paraffin, aluminum stearate, cetostearyl alcohol, propylene glycol, polyethylene glycols, woolfat, hydrogenated lanolin, beeswax, and the like.

Lotions may be formulated with an aqueous or oily base and will, in general, also include one or more of the following: stabilizing agents, emulsifying agents, dispersing agents, suspending agents, thickening agents, coloring agents, perfumes, and the like. A preferred composition of the invention is a lotion containing a PADPRP inhibitor, such as arachidonic acid, linoleic acid, oleic acid, and/or nicotinamide, that is applied topically to treat skin aging. A more potent version of the lotion would include a PADPRP inhibitor such as 1, 5-DHI. Powders may be formed with the aid of any suitable powder base, *e.g.*, talc, lactose, starch, and the like. Drops

may be formulated with an aqueous base or non-aqueous base also comprising one or more dispersing agents, suspending agents, solubilizing agents, and the like.

The topical pharmaceutical compositions according to this invention may also include one or more preservatives or bacteriostatic agents, *e.g.*, methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chlorides, and the like. The topical pharmaceutical compositions also can contain other active ingredients such as antimicrobial agents, particularly antibiotics, anesthetics, analgesics, and antipruritic agents.

The compounds of the present invention can also be delivered through mucosal membranes. Transmucosal (i.e., sublingual, buccal, and vaginal) drug delivery provides for an efficient entry of active substances to systemic circulation and reduces immediate metabolism by the liver and intestinal wall flora. Transmucosal drug dosage forms (*e.g.*, tablet, suppository, ointment, pessary, membrane, and powder) are typically held in contact with the mucosal membrane and disintegrate and/or dissolve rapidly to allow immediate systemic absorption.

For delivery to the buccal or sublingual membranes, typically an oral formulation, such as a lozenge, tablet, or capsule will be used. The method of manufacture of these formulations is known in the art, including but not limited to, the addition of the pharmacological agent to a pre-manufactured tablet; cold compression of an inert filler, a binder, and either a pharmacological agent or a substance containing the agent (as described in U.S. Patent No. 4,806,356); and encapsulation. Another oral formulation is one that can be applied with an adhesive, such as the cellulose derivative, hydroxypropyl cellulose, to the oral mucosa, for example, as described in U.S. Pat. No. 4,940,587. This buccal adhesive formulation, when applied to the buccal mucosa, allows for controlled release of the pharmacological agent into the mouth and through the buccal mucosa.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Thus, this invention provides compositions for intravenous administration which comprise a solution of the PADPRP inhibitor dissolved or suspended in an acceptable carrier. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, buffered water, saline, dextrose, glycerol, ethanol or the like. These compositions will sometimes be sterilized by conventional, well known, sterilization techniques, or can be sterile filtered. The resulting solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

Another approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained. See, *e.g.*, U.S. Patent No. 3,710,795, which is incorporated herein by reference.

Liquid pharmaceutically administerable compositions can, for example, be prepared by dissolving, dispersing, etc., an active compound as defined above and optional pharmaceutical adjuvants in a excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, olive oil and other lipophilic solvents, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's, supra*. The composition or formulation to be administered will, in any event, contain an effective amount of the active compound.

For solid compositions, conventional nontoxic solid carriers can be used, which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 0.1-95% of active ingredient, preferably about 20%.

The compositions containing the compounds can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, as described above, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective amount or dose." Amounts effective for this use will depend on the severity of the disease and the weight and general state of the patient.

Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment can cease. Patients can, however, require intermittent treatment on a long-term basis upon any recurrence of the disease symptoms.

In prophylactic applications, compositions containing the compounds of the invention are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective amount or dose." In this use, the precise amounts again depend on the patient's state of health and weight.

With regard to the prophylactic applications of the present invention, the present invention provides useful methods to delay the progression of AIDS. While not wishing to be bound by theory, the present inventor believes that immune cell senescence may induce HIV expression, which is known to be increased upon DNA damage (see, *e.g.*, Valerie *et al.*, 1988, *Nature 333*:78-81, and Stein *et al.*, 1989, *Mol. Cell. Biol. 9*:5169-5181). One can therefore administer a

PADPRP inhibitor according to the method of the invention to those infected with HIV to delay the onset of AIDS.

One can also employ a novel screening method of the invention to find more effective inhibitors of PADPRP and to find any compound that delays the onset of senescence and/or delays expression of HIV in HIV-infected cells. In this method, one first constructs a recombinant vector that comprises the HIV long terminal repeat (LTR) promoter positioned to drive expression of a reporter gene product (a reporter gene is a gene that produces a gene product that can be readily assayed, i.e., the beta-galactosidase gene, the alkaline phosphatase gene, etc.). This vector is used to transform suitable cell lines, such as eukaryotic fibroblast or lymphocyte cell lines, which when grown to senescence should show induction of expression of the reporter gene. In the screen, one assays whether test compounds delay, inhibit, or prevent the induction of expression of the reporter gene caused by senescence. Compounds that could delay senescence would delay, inhibit, or prevent the induction of expression of the reporter gene and would be useful for purposes of the present invention not only generally to prevent cell senescence but also specifically to treat AIDS.

The foregoing text describes various aspects of the invention and how the invention can be practiced. The description of the invention is not intended to provide an exhaustive description of the many different embodiments of the invention. All publications and patent applications cited above are hereby incorporated herein by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

## Examples

The following examples demonstrate how PADPRP inhibitors can be used to alter senescence-related gene expression and also provide methods for identifying compounds that alter senescence-related gene expression. The examples should not be construed as limiting the invention, as the examples merely provide specific alternative methods useful in understanding and practicing the invention.

### Example 1

### Measuring Altered Gene Expression in (mRNA) Senescent Cells

Human fibroblast BJ cells, at Population Doubling (PDL) 94, were plated in regular growth medium and then changed to low serum medium to reflect more physiological conditions as described by Linskens, *et al.*, 1995, *Nucleic Acids Res. 23*, No. 16:3244-3251 (1995). The medium was DMEM/199 supplemented with 0.5% bovine calf serum. The cells were treated daily for 13 days with the PADPRP inhibitor 1,5-dihydroxyisoquinoline (100 µM). The control cells were treated with and without DMSO (dimethylsulfoxide), the solvent used in the drug treated cells to administer the PADPRP inhibitor. In addition, old and young control cells which were not treated with DMSO were tested for comparison. RNA was prepared from the treated and control cells according to the techniques described in PCT Publication No. 96/13610, *supra*, and Northern

blotting was conducted. Probes specific for senescence-related genes were analyzed, and treated and control cells were compared. The results are shown in Table 1, in which gene expression is normalized in each row, with one control (of the three) showing the lowest level of gene expression arbitrarily set at 1 to provide basis for comparing expression (in this test) levels of a specific gene in one cell with the levels of that same gene in another cell. Three genes that are particularly relevant to age-related changes in skin are collagen Ia1, collagenase, and elastin (West, 1994. *Arch. Derm. 130*:87-95). As can be seen from Table 1, elastin expression of the treated cells was significantly increased in the 1,5- dihydroxyisoquinoline treated senescent cells relative to the control cells. Elastin expression is significantly higher in young cells (16.0 in young BJ cells, PDL 38, without DMSO) compared to senescent cells (1.4 in senescent BJ cells, PDL 93, without DMSO). Thus, treatment with 1,5- dihydroxyisoquinoline caused elastin expression levels in senescent cells to change to levels similar to those found in much younger cells. Similarly, a beneficial effect is seen in collagenase and collagen Ia1 expression with 1,5- dihydroxy-isoquinoline an effect that is improved using the DMSO vehicle.

Table 1: Changes in Gene Expression upon Treatment of Senescent Cells with 1,5-Dihydroxyisoquinoline .

| Probe | Young BJ 38 No DMSO 0.5% Serum | Old BJ 93 No DMSO Control | Old BJ 94 DMSO Control[a] | Old BJ 94 1,5-dihydroxy-isoquinoline |
|---|---|---|---|---|
| Collagen Ia1 | 3.5 | 1.0 | 1.7 | 2.0 |
| ALDH 1 | 3.2 | 1.0 | 1.0 | 1.6 |
| LAMININ A | 3.4 | 1.4 | 1.0 | 1.2 |
| ELASTIN | 16 | 1.4 | 1.0 | 6.0 |
| EPC-1 | 1.0 | 1.7 | 1.2 | 0.7 |
| 80KL | 2.0 | 1.0 | 1.3 | 1.3 |
| COLLAGENASE | 1.2 | 19.2 | 1.0 | 0.4 |
| PAI-1 | 1.0 | 3.3 | 5.5 | 6.6 |
| UPA | 1.0 | 10.0 | 3.3 | 3.5 |
| PAI-11 | 1.0 | 4.1 | 1.7 | 3.0 |

a. normalized values

Example 2

Measuring Altered Gene Expression (Protein) in Senescent Cells

Approximately, $10^5$ BJ cells at PDL 95-100 were plated and grown in 15 cm dishes. The growth medium was DMEM/199 supplemented with 10% bovine calf serum. The cells were treated daily for 24 hours with the PADPRP inhibitor (100 µg / 1 mL of medium) 1,5-dihydroxyisoquinoline. The cells were washed with phosphate buffered solution (PBS), then permeablized with 4% paraformaldehyde for 5 minutes, then washed with PBS, and treated with 100% cold methanol for 10 minutes. The methanol was removed, and the cells were washed with PBS, and then treated with 10% serum to block nonspecific antibody binding. About 1 mL of the appropriate commercially available antibody solutions (1:500 dilution, Vector) was added to the cells and the mixture incubated for 1 hour. The cells were rinsed and washed three times with PBS. A secondary antibody, goat anti-mouse IgG (1 mL), with a biotin tag was added along with 1 mL of a solution containing streptavidin conjugated to alkaline phosphatase and 1 mL of NBT reagent (Vector). The cells were then washed and changes in gene expression were noted colorimetrically.

In one embodiment, four senescence-specific genes (collagen I, collagen III, collagenase, and interferon gamma) in senescent cells treated with 1,5-dihydroxyisoquinoline were monitored, and the results showed a decrease in interferon gamma expression with no observable change in the expression levels of the other three genes, demonstrating that PADPRP inhibitors can alter senescence-specific gene expression.

The reagents employed in the examples are commercially available or can be prepared using commercially available instrumentation, methods, or reagents known in the art. The foregoing examples illustrate how PADPRP inhibitors can alter senescent gene expression and provide methods to determine additional compound that alter senescent gene expression. The examples are not intended to provide an exhaustive description of the many different embodiments of the invention. Thus, although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, those of ordinary skill in the art will realize readily that many changes and modification can be made thereto without departing from the spirit or scope of the appended claims.

We Claim:

1. A method to extend the lifespan and proliferative capacity of cells, which method comprises administering a therapeutically effective amount of a PADPRP inhibitor to said cells under conditions such that PADPRP activity is inhibited.

2. The method of Claim 1, wherein said cells are in *in vitro* culture.

3. The method of Claim 1, wherein said cells are *in vivo*, and said method is used to treat a disease or disease conditions induced or exacerbated by cellular senescence.

4. The method of Claim 3, wherein said disease is a disease selected from the group consisting of skin aging, Alzheimer's disease, atherosclerosis, osteoarthritis, osteoporosis, muscular dystrophy, age-related macular degeneration, immune senescence, and AIDS.

5. The method of Claim 1, wherein said inhibitor is selected from the group of inhibitors consisting of 3-hydroxybenzamide, 3-acetamidobenzamide, 3-methoxybenzamide, 3-methylbenzamide, 3-fluorobenzamide, 2-methoxybenzamide, 3-chlorobenzamide, benzamide, 4-amino-1,8-naphthalimide, 2H-benz[c]isoquinolin-1-one [6(5H)-phenanthridinone], 2-nitro-6(5H)-phenanthridinone, 1,5-dihydroxyisoquinoline, 2H-benz[de]isoquinoline-1,3-dione, (1,8-naphthalimide), 2-methyl-4(3H)-quinazolinone, 1-hydroxyisoquinoline (isocarbostyril), 2,4(1H,3H)-quinazolinedione (benzoyleneurea), chlorthenoxazin, 4-hydroxyquinazoline, 1(2H)-phthalazinone, 2-phenylchromone (flavone), and 3-aminophthalhydrazide (luminol).

6. The method of Claim 1, wherein said inhibitor is 3-hydroxybenzamide.

7. The method of Claim 1, wherein said inhibitor is 3-aminobenzamide.

8. A pharmaceutical formulation comprising a PADPRP inhibitor in a therapeutically or pharmaceutically effective dose together with one or more pharmaceutically or therapeutically acceptable carriers.

9. The pharmaceutical formulation of Claim 8, wherein said inhibitor is selected from the group of inhibitors consisting of 3-hydroxybenzamide, 3-acetamidobenzamide, 3-methoxybenzamide, 3-methylbenzamide, 3-fluorobenzamide, 2-methoxybenzamide, 3-chlorobenzamide, benzamide, 4-amino-1,8-naphthalimide, 2H-benz[c]isoquinolin-1-one [6(5H)-phenanthridinone], 2-nitro-6(5H)-phenanthridinone, 1,5-dihydroxyisoquinoline, 2H-benz[de]isoquinoline-1,3-dione, (1,8-naphthalimide), 2-methyl-4(3H)-quinazolinone, 1-hydroxyisoquinoline (isocarbostyril), 2,4(1H,3H)-quinazolinedione (benzoyleneurea), chlorthenoxazin, 4-hydroxyquinazoline, 1(2H)-phthalazinone, 2-phenylchromone (flavone), nicotinamide, and 3-aminophthalhydrazide (luminol).

10. A method to alter gene expression of senescent cells, which method comprises administering a therapeutically effective amount of a PADPRP inhibitor to said cells under conditions such that PADPRP activity is inhibited.

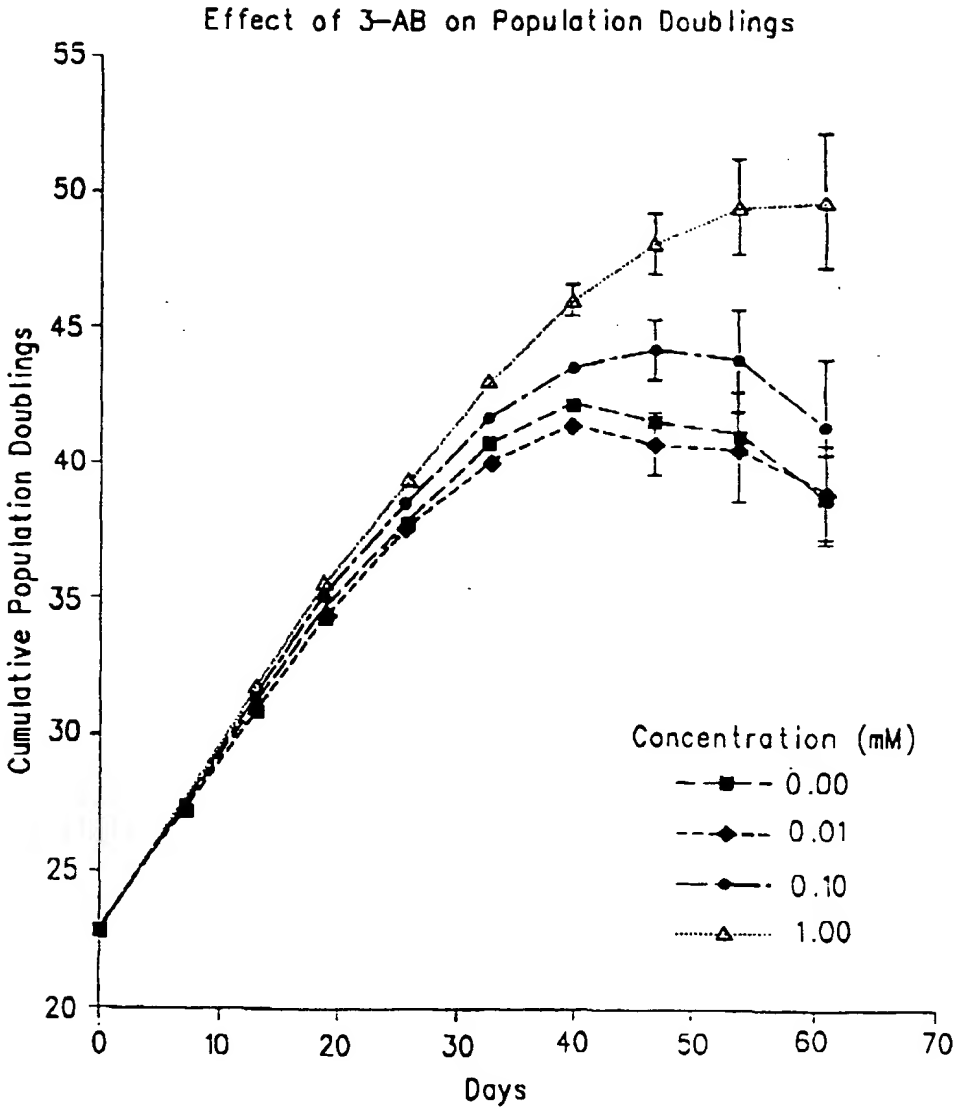
Effect of 3-AB on Population Doublings
Cumulative Population Doublings
55
50
45
40
35
30
25
20
0
10
20
30
40
50
60
70
Days
Concentration (mM)
0.00
0.01
0.10
1.00

FIG. 1

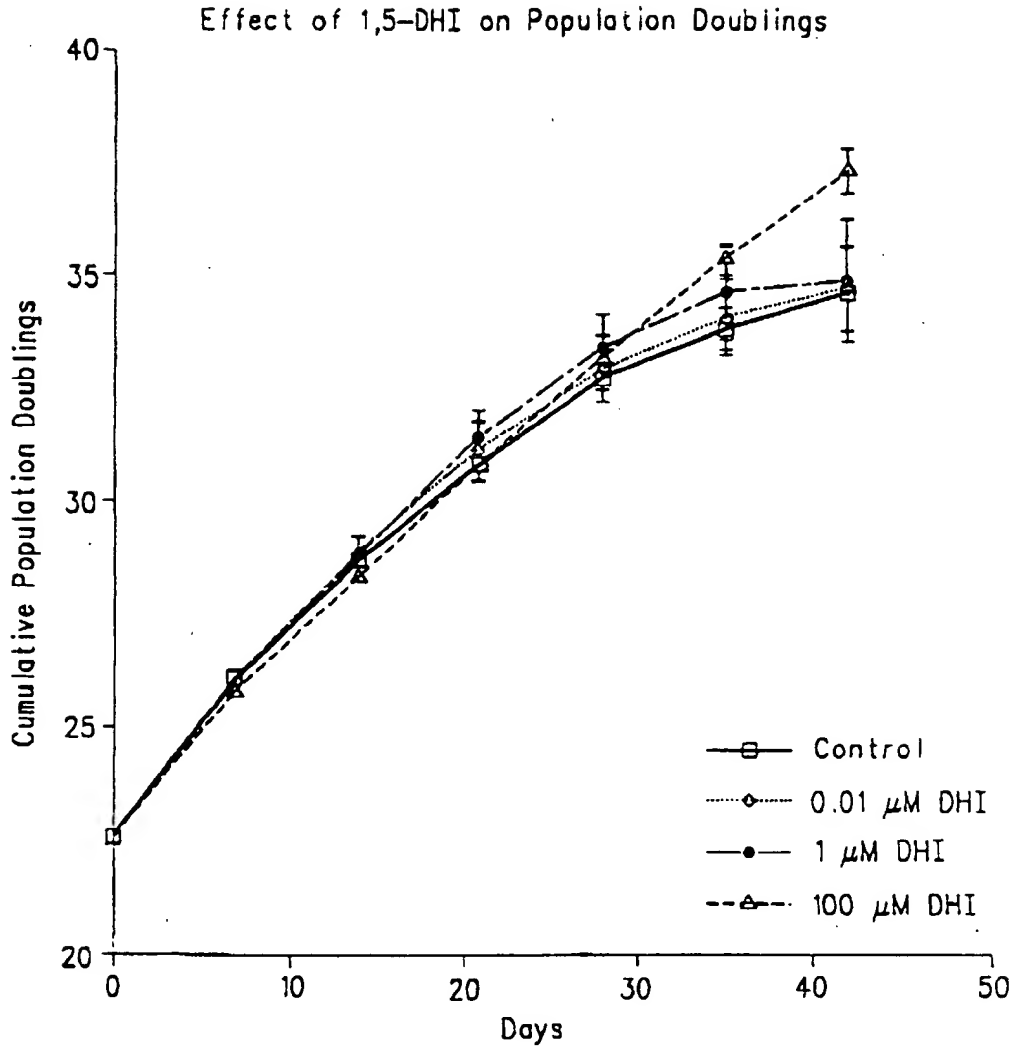
Effect of 1,5-DHI on Population Doublings
Cumulative Population Doublings
40
35
30
25
20
0
10
20
30
40
50
Days
Control
0.01 μM DHI
1 μM DHI
100 μM DHI

FIG. 2

# INTERNATIONAL SEARCH REPORT

Intern al Applicaoo No
PCT/US 96/20630

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/165 A61K31/47 A61K31/475 A61K31/505 A61K31/535 A61K31/35 A61K31/185

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
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| X | GB 2 281 860 A (CONSULTANTS SUPPLIERS LIMITED) 22 March 1995 see page 2 see page 12 --- | 8,9 |
| X | WO 86 06628 A (STANFORD RES INST INT) 20 November 1986 see page 8 - page 11 --- | 8,9 |
| X | US 5 215 738 A (LEE WILLIAM W ET AL) 1 June 1993 see column 4 - column 6 --- -/-- | 8,9 |

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 8 September 1997 | 24.09.97 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. ( + 31-70) 340-2040, Tx. 31 651 epo nl, Fax ( + 31-70) 340-3016 | Trifilieff-Riolo, S |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 96/20630

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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| X | J. BIOL. CHEM.,<br>vol. 267, 1992,<br>pages 1569-1575, XP002040060<br>BANASIK ET AL: "specific inhibitors of padpr synthetase..."<br>cited in the application<br>see the whole document | 8,9 |
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/US 96/20630

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1,3-7,10 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 5-7
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds mentioned in the examples of the description.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ..al Application No
PCT/US 96/20630

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